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Role of Bioactivation in Drug-Induced Hypersensitivity Reactions

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ABSTRACT

Drug-induced hypersensitivity reactions are a major problem in both clinical treatment and drug development. This review covers recent developments in our understanding of the pathogenic mechanisms involved, with special focus on the potential role of metabolism and bioactivation in generating a chemical signal for activation of the immune system. The possible role of haptenation and neoantigen formation is discussed, alongside recent findings that challenge this paradigm. Additionally, the essential role of costimulation is examined, as are the potential points whereby costimulation may be driven by reactive metabolites. The relevance of local generation of metabolites in determining the location and character of a reaction is also covered.

KEYWORDS: Hypersensitivity, drug metabolism, bioactivation, sulfamethoxazole

INTRODUCTION

Adverse drug reactions are a common clinical problem that can often compromise good patient care. A large-scale meta-analysis estimated that adverse drug reactions occur in as many as 15% of all hospital patients,[1] although this estimate has been widely disputed.[2] In a more recent prospective study in the United Kingdom, the proportion of hospital admissions directly caused by adverse drug reactions was 5.2%.[3] Most of these reactions (76.2%-95%) were defined as Type A (dose-dependent) reactions by the definition of Rawlins and Thompson,[4] while the remainder were Type B (idiosyncratic).

Idiosyncratic reactions are a major cause of drug withdrawal both late in drug development and at the postmarketing stage. Such idiosyncratic reactions contribute to the high level of attrition that is presently encountered in drug development, either early in development as a result of crude screening techniques, or when such reactions are identified in clinical trials. It is therefore imperative that we understand the fundamental mechanisms involved in idiosyncratic reactions.

Hypersensitivity reactions are idiosyncratic reactions that involve activation of a pathogenic, drug-specific immune response. However, because of obvious difficulties in determining cause in clinical practice, the term is generally used to describe adverse drug reactions with concurrent fever, rash, and/or internal organ involvement.[5] This extends from minor rashes to severe, potentially fatal reactions such as Stevens-Johnson syndrome and toxic epidermal necrolysis. In this review we will concentrate particularly on T-cell-mediated reactions, although immunoglobulin E (IgE)-mediated and IgG-mediated reactions are also of clinical importance, particularly for penicillins.[6]

MECHANISMS OF DRUG ANTIGEN-SPECIFIC T-CELL ACTIVATION

Drug antigen-specific T cells have been isolated, cloned, and characterized from hypersensitive patients in terms of their cellular phenotype and functionality.[7,8] Isolated T cells can express either the CD4 or the CD8 coreceptor, or both. Drug stimulation results in secretion of high levels of polarizing cytokines,[9-16] and Fas ligand (FasL) or perforin-mediated killing of autologous keratinocytes.[17,18] Furthermore, drug-specific CD8+ T cells have also been identified and characterized in blister fluid taken from patients with toxic epidermal necrolysis.[19,20] A major focus of this review is to describe our current understanding of the mechanisms of T-cell activation in terms of the nature of the antigen presented and the role of metabolic drug activation.

In 1935, Landsteiner and Jacobs undertook a series of seminal studies on the sensitization potential of low-molecular-weight organic compounds.[21] They found a strong correlation between the sensitization potential in vivo and the protein reactivity in vitro. These findings have formed the basis of the hapten hypothesis, which posits that drugs—or more commonly, reactive metabolites formed by the normal processes of metabolizing enzymes—are recognized by only drug-specific T cells following haptenation to self-proteins.[22] This leads to formation of a neoantigen that can be recognized by T cells to override self-tolerance, and induction of a potentially pathogenic immune response. This mechanism

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has been well documented for contact sensitizers such as dinitrochlorobenzene[23,24] and respiratory allergens such as trimellitic anhydride.[25,26] More speculatively, it is thought to be a mechanism for the breaking of self-tolerance in autoimmunity.[27-29] However, until recently there has been little direct evidence for its importance in drug hypersensitivity.

Recent studies exploring mechanisms of drug hypersensitivity have focused on sulfamethoxazole, because it is known to cause hypersensitivity and because much is known about its disposition in the body. As such, sulfamethoxazole will be used as an example throughout this review (Figure 1 depicts our current understanding of sulfamethoxazole metabolism and the role of metabolic drug activation in the generation of antigen-specific T cells in sulfamethoxazole hypersensitivity). Sulfamethoxazole is metabolized by CYP2C9 in human liver to a proreactive hydroxylamine metabolite.[30-33] Sulfamethoxazole hydroxylamine is spontaneously converted to nitroso sulfamethoxazole,[34,35] which is unstable and reacts with the hydroxylamine to generate azo and azoxy dimers. Further oxidation can also generate nitro sulfamethoxazole.[36] Importantly, reduction of nitroso sulfamethoxazole can occur either via interaction with nonprotein thiols (eg, glutathione) and ascorbate, or enzymatically.[30,37] Thus, the critical balance between metabolic activation and detoxification in a given cell system ultimately determines the level of exposure to nitroso sulfamethoxazole. It is therefore interesting to note that thiol and ascorbate deficiencies have been reported with HIV infection,[38-40] which may thereby lead to a decreased capacity to reduce nitroso sulfamethoxazole[38] and an increased metabolite-mediated lymphocyte toxicity,[41] alongside a greatly increased risk of hypersensitivity reactions.[42] However, viral infection will also have significant immunological effects unrelated to metabolite detoxification, such as deranged regulatory mechanisms.[43] Additionally, some studies have failed to find a link,[44] so this remains controversial.

Nitroso sulfamethoxazole binds covalently to cellular proteins.[45-49] Such binding above a threshold level may be responsible for the direct toxic effects of sulfamethoxazole.[36,49] The intrinsic instability of nitroso sulfamethoxazole suggests that localized generation and covalent binding, both in skin or in antigen-presenting cells, will render proteins immunogenic and therefore be the source of the ultimate antigenic determinant. T cells isolated from patients hypersensitive to sulfamethoxazole and structurally unrelated drugs, as well as animal models of immunogenicity, have been used to explore the nature of the interaction between chemicals and immunological receptors. T cells can be stimulated by either (1) protein-reactive electrophilic metabolites bound directly to major histocompatibility complex (MHC) via a stable covalent bond,[50,51] or (2) processed peptides derived from conjugated protein.[10,36]

It is also worth pointing out that almost all drugs associated with a comparatively high incidence of hypersensitivity reactions are known to form reactive metabolites. It is not known whether this is simply observational bias, due to the increased research focus on these drugs, or is of genuine importance.

Recently, an alternative hypothesis known as the P-I concept has been proposed. The P-I concept posits that drugs can activate T cells directly in the absence of metabolism, covalent binding, and processing,[52] through a reversible interaction between the T-cell receptor, MHC, and the drug. This has been unquestionably demonstrated using T-cell clones from patients ex vivo for several drugs, including sulfamethoxazole,[13] lidocaine,[53] carbamazepine,[9] lamotrigine,[11] and phenindione,[10] but there is as yet little evidence that this complex is sufficient to induce a primary immune response. Criticisms of these findings have been based on the requirement for in vitro expansion of drug-specific T cells prior to cloning, which could affect the apparent makeup of drug-specific T cells. Recently, this point was addressed by Nassif et al,[19] who identified parent drug–specific T cells from blister fluid in toxic epidermal necrolysis patients. However, their interpretations regarding the lack of a role for metabolism remain controversial since blister fluid T cells from *all* patients also responded to nitroso sulfamethoxazole.[54] Engler et al[55] successfully induced a primary T-cell immune response in vitro against sulfamethoxazole using blood from 3 of 10 healthy individuals previously not exposed to the drug. However, nitroso sulfamethoxazole-specific cytotoxic T-cell responses were detected using blood from 9 of 10 of the same healthy volunteers; thus, the question as to whether non–covalently bound drug or covalently bound metabolite stimulates pathogenic T cells has not been fully elucidated and warrants further investigation. A particular area that has not been studied previously is the ability of peptides derived from drug-metabolite modified cutaneous or immune cell protein to stimulate T cells from hypersensitive patients.

Of course, the apparently competing hypotheses described above are by no means mutually exclusive. For instance, since sulfamethoxazole and nitroso sulfamethoxazole-specific T cells coexist in all patients studied so far,[51] metabolism and haptenation may be required for initiation of an immune response, and during the response avidity spreading occurs to the continually present parent drug.

Danger Signaling, Drug Metabolism, and Drug Hypersensitivity

The danger hypothesis, postulated by Polly Matzinger[56] as an extension of Charles Janeway's work on the links between the innate and the adaptive immune systems,[57] holds that the nonself nature of a foreign antigen is not what induces an immune response; instead, it is "danger signals," such as cell damage or infection, that activate the immune system.

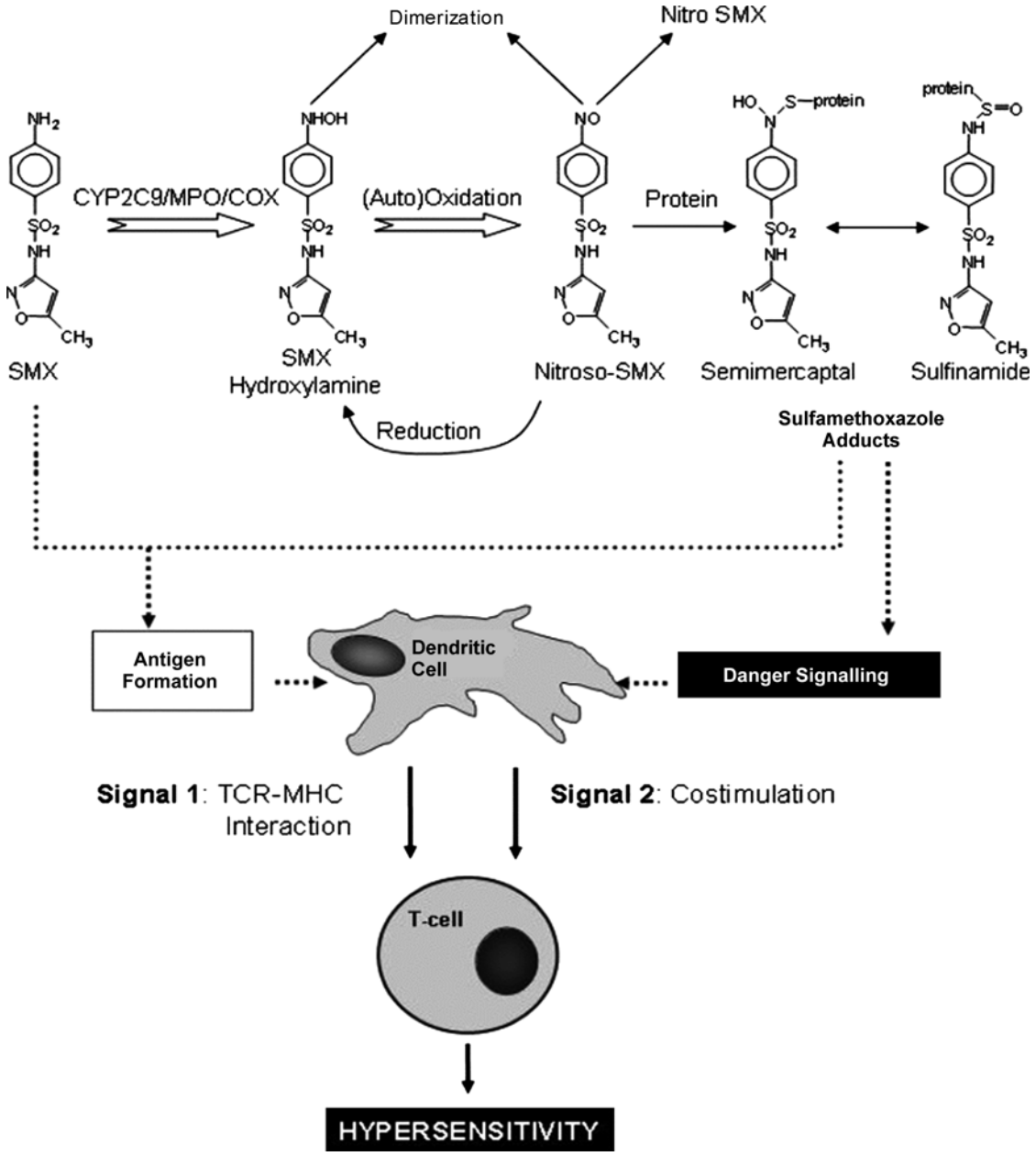

Figure 1. Under our current understanding, sulfamethoxazole can induce a hypersensitivity reaction by acting as an antigen, either as the parent drug or as a protein conjugate. Additionally, the reactive metabolite can cause cell damage, leading to danger signaling and activation of costimulatory pathways. CYP indicates cytochrome P450; MPO, myeloperoxidase; COX, cyclooxygenase; SMX, sulfamethoxazole; TCR, T-cell receptor; MHC, major histocompatibility complex.

Therefore, it can be said that effective activation of the adaptive immune system requires 2 signals[58]: signal 1, which is the T-cell-receptor-mediated recognition of an MHC-restricted antigen; and signal 2, which represents the interactions between various costimulatory ligands and receptors between the T cell and the antigen-presenting cell, such as CD28:CD86 and CD40:CD154. In the absence of signal 2, signal 1 simply leads to tolerance, either by anergy or by apoptosis of responding T cells.[59] Danger signals act via this signal 2 pathway, by upregulating costimulatory markers on professional antigen-presenting cells, such as dendritic cells. Several studies have focused on the ability of stressed, dead, or dying cells to provide maturation signals to dendritic cells. Initial studies revealed that cells killed necrotically, but not viable or apoptotic cells, activate dendritic cells.[60] Heat shock proteins released from dying cells are obvious candidates as danger-signaling molecules, and their ability to provide maturation signals to dendritic cells has been discussed in detail elsewhere.[61] More recently, a groundbreaking study by Shi et al[62] used chemical analyses to define uric acid crystals as potent messengers that are released by injured and dying cells and that can stimulate dendritic cell maturation and enhance CD8+ T-cell responses in vivo.

When considering the potential for drug metabolites to interact with dendritic cells and provide maturation signals, lessons can be learned from studies with contact sensitizers, which because of their intrinsic protein reactivity are often toxic to immune cells at relatively low concentrations. This toxicity can be the result of covalent binding, either to specific target proteins, for instance Keap1,[63] or nonspecifically, potentially exposing hydrophobic residues and activating toll-like receptors.[64] Alternatively, this toxicity could be due to the generation or impaired detoxification of reactive oxygen species, a major factor in activation of the proinflammatory NF-kB pathway,[65] which is essential for complete dendritic cell maturation.[66,67] It has been suggested that dendritic cell maturation may be due to inactivation of thioredoxin reductase and consequent impaired detoxification of reactive oxygen species.[68] However, this direct activation is inhibited by thiol antioxidants[69] but not other antioxidants, suggesting that the effect of thiol antioxidants is not solely due to radical scavenging. This idea is supported by the finding that glutathione depletion enhances this activation.[70] A wide variety of structurally unrelated contact sensitizers have been found to provoke signal 2 via direct activation of dendritic cells and monocytes, as determined by either upregulation of cell surface markers, particularly CD80, CD86, CD40, HLA-DR,[71-74] or chemokine receptors[75]; by activation of signal transduction pathways[69,76-79]; or by functional effects, for instance, enhanced activation of allogeneic T cells[72,80,81] or in vivo sensitization.[82] Recently, Hulette et al[83] showed that contact sensitizers stimulate dendritic cell maturation only at concentrations associated with low levels of cell death, presumably via a classical "danger" response[56] and the recognition of released endogenous signals.[60,62] However, in recent unpublished experiments, using the hair dye allergen p-phenylenediamine as a paradigm, we have shown that dendritic cell maturation can be induced at nontoxic concentrations, as measured by increased expression of CD40 and stimulation of allogeneic lymphocyte proliferation. CD40 receptor ligand binding is known to stimulate an important signaling pathway that results ultimately in further dendritic cell maturation.[84] In addition, abnormal CD40 signaling in certain autoimmune diseases is thought to contribute to disease progression,[85,86] and as such, the focus of our current research is to determine whether CD40 signaling is related to susceptibility to p-phenylenediamine sensitization.

Based on the observation that many protein-reactive drug metabolites bind covalently to thiol-rich protein, it is not a great leap to imagine that a similar effect may be driven by drug metabolites, although there is no published evidence that this occurs.

Several studies indicate a trend toward higher drug metabolite-mediated toxicity to immune cells from patients with drug hypersensitivity.[87-90] However, whether these observations reflect differences in cellular metabolism, the extent or site of protein binding, or modulation of intracellular defense pathways is not known.

In unpublished experiments, we have shown the presence of sulfamethoxazole metabolite-modified intracellular proteins when sulfamethoxazole was incubated in vitro with dendritic cells. Dendritic cell metabolism of sulfamethoxazole resulted in covalent modification of endogenous protein and subsequent increased expression of the dendritic cell costimulatory receptor CD40. When mice were administered nitroso sulfamethoxazole in the presence of an anti-CD40 ligand-blocking antibody, drug metabolite-specific T-cell proliferation was completely inhibited. Thus, the CD40 signaling pathway seems to be important in the development of sulfamethoxazole immunogenicity. Further studies are underway to explore the effects of sulfamethoxazole treatment on dendritic cells from hypersensitive patients to evaluate whether altered immune cell metabolism and dendritic cell activation are associated with individual susceptibility.

Location of Metabolic Drug Activation and Its Relevance to Drug Hypersensitivity

The following section provides a brief overview of the importance of organ/tissue-specific metabolic drug activation in the development of drug hypersensitivity in some individuals but not others. Because space is limited, we have focused on metabolism in the liver, the skin, and the immune system. Although the liver is known to be the most important location for drug metabolism in the body, extrahepatic metabolism is also suspected to have an important role in the induction of hypersensitivity reactions. Table 1 shows the relative abundance of certain enzymes in a given cell type.

Liver

Quantitatively, the liver is the most important organ for drug metabolism. Hepatic metabolism, primarily via cytochromes P450 (CYP), is the main route of bioactivation for drugs that have been linked to hypersensitivity, such as sulfamethoxazole,[100] carbamazepine,[101,102] phenytoin,[103] abacavir,[104] and halothane.[105] In most (although not all) cases, the reactive species formed are so reactive that they are unlikely to survive long in circulation, which implies that the liver will receive much greater exposure from reactive metabolites than other tissues. However, despite the increased exposure, the liver is rarely the main target for antigen-specific T cells. There are 2 likely reasons for this discrepancy. First, the liver is very well protected from toxic insult[106] by cellular cytoprotective measures, such as high glutathione and N-acetylcysteine levels, and readily activates further defenses via Nrf2 and NF-kB driven transcription. Second, the

Table 1. Relative Expression of Several Xenobiotic Metabolizing Enzymes in Different Cell Types*

	Cytochrome P450												
	1A1	**1A2**	**1B1**	**2B6**	**2C9**	**2C19**	**2D6**	**2E1**	**3A4**	**3A5**	**MPO**	**COX**	**Refs**
Hepatocytes	+	++	+	+	++	+	+	++	+++	+++	ND	ND	91
Keratinocytes	+	ND	+	+	†	†	+	+	—	+	ND	ND	92,93
Lymphocytes	+	—	++	ND	ND	ND	++‡	++	+	ND	+	+	94,95
Dendritic cells	+	+	+++	+	+	+	+	+	—	+	—	+	96-98

*MPO indicates myeloperoxidase; COX, cyclooxygenase; ND, not determined; CYP, cytochrome P450. +/++/+++ indicate relative expression within a given cell type. These are not intended to be used for comparison between different cell types.
†CYP2C family enzymes present,[99] although no data are available on individual enzymes.
‡High expression but possibly a truncated inactive form.

liver is an immunologically privileged organ,[107] and hepatic activation of T cells by Kupffer cells is likely to lead to tolerance rather than a pathogenic immune response. This is believed to be at least partly due to increased expression of FasL in nonlymphoid hepatic tissue,[108] which will drive T cells to apoptosis rather than activation.

Halothane hepatitis is well studied as a model of drug-induced immune-mediated hepatotoxicity. A transient increase in transaminases is seen in up to 20% of patients, whereas a severe reaction, characterized by massive cell necrosis, occurs in ~1 patient per 35 000 on primary exposure, and 1 in 3700 on secondary exposure.[109] Halothane is metabolized in the liver, predominantly by CYP2E1,[110] to trifluoroacetic acid, chloride, and bromide.[111] However, the reactive metabolite trifluoroacetyl chloride is also formed, which readily forms trifluoroacetyl adducts to free amino groups on hepatic proteins.[112] The role of metabolism in the hepatitis associated with halothane administration is best illustrated by a global consideration of the relationship between the in vivo metabolism of general anesthetics and the observed incidence of adverse drug reactions in humans. Up to 50% of methoxyflurane and halothane is excreted as metabolites in human urine, and their administration is associated with severe toxicities. In contrast, less than 3% of enflurane and isoflurane is excreted as urinary metabolites, and human exposure is only rarely associated with hepatotoxicity.[6]

Antibodies to adducted neoantigens, particularly certain microsomal proteins,[113] have been identified in the sera of halothane hepatitis patients,[114] which has led some to conclude that these adducts are immunologically relevant. However, similar adducts are found in nonhypersensitive halothane-exposed patients, where they do not appear to be pathogenic,[113] indicating that the major determinant of response may be idiosyncrasies in the immune system rather than generation of reactive metabolites and adducts. Additionally, although cellular reactivity to halothane has been identified both in humans[115] and in a guinea pig model,[116,117] most studies have concentrated on the role of autoantibodies and antibodies to neoantigens. It is therefore possible that an important pathogenic mechanism is being overlooked, and further work is required in order to address this.

Skin

Recently, there has been a lot of interest in the metabolic potential of the skin,[118] as skin is the most common site of hypersensitivity reactions (although it must be noted that we do not know how many minor hypersensitivity reactions are associated with subclinical internal organ damage). Keratinocytes are metabolically active, expressing high levels of several CYP isoforms.[92,119] CYP messenger RNA has also been identified in other skin cell types, such as fibroblasts and melanocytes.[99] When the activity of primary keratinocytes is compared with primary liver tissue,[118] conflicting data have been obtained, which is likely to be due to the wide inter- and intraindividual variation in both skin and hepatic CYP expression. Interestingly, although most important hepatic CYPs are expressed in the skin (including CYPs 1A1, 1B1, 2B6, 2C9, and 3A4), there are several CYPs which are much more abundant in the skin, including several members of the CYP2 family that have never been identified in the liver.[93] The relevance of this for hypersensitivity is not known, but it is possible that the relative proportion of metabolites produced in the skin may differ from those in the liver, with possible immunotoxicological implications.

One of the most important recent findings in this area was the demonstration that primary keratinocytes are capable of oxidative metabolism of sulfamethoxazole to its corresponding hydroxylamine metabolite,[48] which readily autooxidizes to a highly protein-reactive arylnitroso species.[35] Intracellular sulfamethoxazole-protein adducts have also been identified in primary human keratinocytes when incubated with sulfamethoxazole, and these adducts colocalize on the cell surface with HLA-ABC.[120] It is not known as yet whether these adducts are actively presented in the context of HLA or are simply colocalized, for instance as part of lipid rafts. Furthermore, the ability of hapten-modified cutaneous protein to stimulate T cells from hypersensitive patients has not been evaluated.

IMMUNE SYSTEM

There is less known about the metabolic activity of cells of the immune system than is known about the metabolic activity of cells of the liver or the skin. CYP expression in peripheral blood lymphocytes has been assessed,[94,121-123] although a wide variability in findings[124] makes interpretation difficult. Other immune cells are not as well studied, but there is some evidence that monocyte-derived dendritic cells[96] and Langerhans cells[99] are metabolically active. A common feature of CYP expression in the immune system is the high levels of expression of CYP1B1,[125] which is not expressed hepatically. Furthermore, studies have shown that this isoform has specificity for several xenobiotics,[126] suggesting that there is the potential for specific immunological activation of drugs.

Although CYP enzymes are the most widely studied in xenobiotic bioactivation, other enzyme systems, particularly peroxidases, are also capable of oxidative metabolism of small molecules. Two of these, myeloperoxidase[127] and prostaglandin-H-synthase,[128] are highly expressed in cells of the immune system, particularly neutrophils and monocytes. Several drugs associated with a relatively high incidence of hypersensitivity reactions—including sulphamethoxazole,[129,130] carbamazepine,[131] dapsone,[130] and trimethoprim[132]—are metabolized to reactive intermediates by these systems. Neutrophils express both peroxidase systems (but low levels of CYPs) and can generate a powerful extracellular oxidizing system when activated. For these reasons, and because of their sheer numbers in circulation, neutrophils have been described as "the greatest drug-metabolising engine outside of the liver."[133] The role of neutrophils in drug-induced lupus has been widely discussed and has been linked to the ability of lupus-inducing drugs to be bioactivated by myeloperoxidase.[134] Additionally, antineutrophil cytoplasm antibodies and autoantibodies to myeloperoxidase have been detected in drug-induced lupus,[135,136] although no evidence has appeared of antibodies to drug-modified proteins. Langerhans cells and dendritic cells express prostaglandin H synthase[97,137] but are negative for myeloperoxidase.[98] Although the specialized antigen-presenting nature and metabolic activity of Langerhans and dendritic cells would presumably enhance their potential for initiating an immune response to haptenated proteins, this has not been unequivocally identified either in vivo or in vitro.

CONCLUSION

Significant progress has been made toward a better understanding of the mechanisms involved in drug hypersensitivity, including the role of oxidative metabolism and reactive metabolites. Paradoxically, however, our increased understanding has cast doubts on the importance of several established hypotheses. Many questions remain to be answered: Is the primary T-cell response to free drug or to drug-protein adducts? Can the T-cell response shift from metabolite to primary drug over the course of a reaction or following recovery? Can the generation of reactive metabolites act as a danger signal to induce a reaction, and is this a major predisposing determinant of individual susceptibility? Can external danger signals (concurrent infection, cell death, etc) increase the risk of a reaction? What proportion of the variation in susceptibility is due to variation in drug metabolism? What role does the extrahepatic generation of metabolites play in determining the location of the reaction?

Answering these questions will require the development of better animal models and innovative in vitro, pharmacogenetic, and clinical studies. Most important, we will need to better understand the connections between different models and various studies. The lessons of the last few years tell us that these tasks will not be easy, but the answers will come.

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